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IMMUNOLOGICAL OBSERVATIONS IN ULCERATIVE CYSTITIS CAUSED BY PSEUDODIPHTHERIA BACILLUS.*

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History of the case.—Woman, typesetter, unmarried, 23 years of age, no history of tuberculosis or hereditary diseases in family; never robust, always of good health except for nervousness and severe pain during menstrual periods. About two years ago began to have a slight vaginal discharge; for this and for the painful menstruation, the uterus was curetted. Did not improve and grew very nervous. Six months later both tubes and ovaries were removed; immediately thereafter intense vaginal pain and a profuse vaginal discharge developed. Gradually she improved and returned to work, but has never been entirely free from distress and discharge since. Six months ago (December, 1907) she was taken with a severe chill followed by painful, frequent, and bloody micturition, tenesmus, fever, and prostration. Since then, better and worse at times. Has lost weight, but worked constantly. No cough or expectoration at any time. A cystoscopic examination by Dr. J. Clarence Webster, to whom I am indebted for the opportunity to study the case, showed a large irregularly ulcerated area, approximately 4×5 cm., upon the posterior wall of the bladder behind the trigone; the base of some of the ulcers were irregular, grayish, and necrotic patches, while others were clean. The ureteral orifices free. The vagina atresic and in the vault was a pocket containing thick pus. This was opened and drained, vagina packed with chynesol gauze. The patient left the hospital temporarily because it was thought unwise to drain the bladder before the vaginal condition improved.

June 8, 1908: Two days after cystoscopic examination; the patient is suffering excruciating pain in the bladder and back associated with a constant desire to urinate, extreme tenesmus, and burning. No distinct evidence of involvement of kidneys or of renal colic can be made out although she complains of severe pain in the back. The pulse is normal; no fever. The 24-hour specimen of urine, 720 c.c., is turbid but not bloody, alkaline, specific gravity 1,015, containing a large amount of albumin but no casts, a few leukocytes and epithelial cells. Stained specimens of sediment show gram-negative, moderately motile bacilli and a fair number of gram-positive bacilli resembling the diphtheria group. The fresh single specimen also shows a large amount of albumin, no casts, some leukocytes showing various stages of disintegration, and a large amount of albuminous débris. Stained specimens of the sediment show about an equal number of the bacilli mentioned.

June 10, 1908: A freshly catheterized specimen was plated out on plain and urine agar. Two varieties of bacilli developed: One gram-negative moderately motile that proved to be the colon bacillus on further cultivation. The other was a gram-positive bacillus showing a rather marked tendency to polymorphism and irregular staining. The same bacilli were again found in the sediment.

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June 15, 1908: Catheterized specimen of urine was thoroughly centrifugated and the sediment was found to contain a fair number of leukocytes, few epithelial cells, no gram-negative bacilli but a rather large number of gram-positive bacilli. The cultures on Löffler's blood serum gave a luxurious growth at the end of 24 hours of the diphtheria-like bacillus. These bacilli were always more numerous in a 24-hour specimen or in a single specimen which had stood for a period. On the other hand the number of leukocytes and epithelial cells was much greater in a fresh specimen than in one which had stood for a time. In order to explain the apparent solution of these cellular elements, some of the urine was made sterile by filtration and used as a culture medium. The pseudodiphtheria bacillus grew rather luxuriantly in it. The urine constantly contained a rather large amount of albumin which presumably furnished the necessary nourishment. A suspension of washed leukocytes and epithelial cells (from another case of cystitis) were now mixed in equal quantity with fresh filtered urine and urine filtered after bacilli had been grown in it for 24 hours, placed in the incubator after determining the number of cells by means of a leukocyte counter and left for 12 and 24 hours. A careful estimate of the number of cells intact at the end of these periods, showed that there was practically no solution of the cells in the urine filtered when fresh, while there was a marked diminution in the urine in which the bacilli had grown for 24 hours.

TABLE 1.

THE EFFECT OF THE URINE UPON LEUKOCYTES AND EPITHELIAL CELLS.

	No. of Leukocytes and Epithelial Cells per c.mm		
	Immediately	12 Hours	24 Hours
Fresh filtered urine	250	225	200
Urine filtered after bacillus had grown in it for 24 hours	325	115	4

Apparently the bacillus growing in the urine produced substances that caused the solution of the cells. A control experiment with normal urine failed because the bacillus did not grow in sufficient amount.

The patient returned to the hospital for further study on June 16, 1908. The temperature during an exacerbation of the bladder symptoms went up to 100° F., otherwise it was normal except for a rise of one degree for several days following June 26, on which day a vesico-vaginal fistula was made. A profuse hemorrhage followed. The drainage of the bladder relieved the suffering, the general condition improved rapidly, and she left the hospital three weeks later. She received 50 million killed bacilli on June 14, 150 million on July 4, and 500 million on July 9, 1908.

Smears from the scrapings from the margin of the ulcerated area showed large numbers of gram-positive bacilli, epithelial cells, and leukocytes. There was no evidence of phagocytosis. Gram-positive bacilli were also present in smears made directly from the granulating surface from the vault of the vagina. Cultures from the scrapings as well as from the vagina yielded the diphtheria-like bacillus in predominating numbers, only a few colon bacilli and staphylococcus colonies developed. Repeated examination of the urine and of the scrapings directly from the ulcerated area in the bladder for tubercle bacilli gave negative results. A conjunctival tuberculin test gave no reaction. Occasional specimens of urine for eight months longer showed a gradual diminution in the number of leukocytes and albumin and a total disappearance of the gram-positive bacilli. The patient's general condition and local improvement were also marked.

The following experiments were made with the bacillus:

Guinea-pig 1, June 15, 1908: Surface growth of one Löffler's blood-serum slant intraperitoneally.

June 16: Very ill. Peritoneal smears rich in leukocytes and bacilli; moderate phagocytosis.

June 20: Lost much in weight. Peritoneal smears still contain gram-positive bacilli and leukocytes.

Gradual recovery. Chloroformed one month later. No lesions.

Guinea-pig 2: Blood-agar slant intraperitoneally. Death in six days. No peritonitis; blood cultures yielded few colonies of the bacillus.

Guinea-pig 3: Blood-agar slant intraperitoneally and 1,000 units of diphtheria antitoxin subcutaneously. Death in 24 hours. Peritoneal and blood smears rich in gram-positive bacilli. Pure culture isolated from both blood and peritoneum.

Guinea-pig 4: June 23, 1908: Surface growth of large plain-agar slant and of Löffler's blood-serum slant intraperitoneally. Grew ill June 29 and died. No bacilli could be found.

It is seen from the results of the experiments that the bacillus was one of moderate virulence and that diphtheria antitoxin failed to protect against the organism as has been the case with other virulent pseudodiphtheria bacilli. Inasmuch as the growth upon various media resembled closely that of diphtheria bacilli especially after they have been cultivated for a time, and for the reason that antitoxin failed to protect, it would seem justifiable to regard the bacillus as a so-called virulent pseudodiphtheria bacillus.[1]

Phagocytic experiments.—In a previous paper[2] I pointed out that leukocytes from pneumonia and other infections may be more active phagocytically than normal leukocytes. The phagocytic activity of leukocytes has been further studied in cases of endocarditis and colon bacillus pyelitis. In these conditions the leukocytes may be more or less active than normally. I have repeatedly observed that while a patient's opsonic index with normal leukocytes may be high, it may be below normal with the homologous leukocytes. In the present case a comparative study of the opsonic power of the patient's serum and the phagocytic activity of the leukocytes was made.

Table 2 illustrates the method by which the results given in the charts were obtained. The experiment was made June 22, 1908. The bloods which furnished the leukocytes were collected in 2 per cent sodium-citrate solution at practically the same time, and washed twice in at least 40 times the amount of isotonic salt solution, this being sufficient to remove the opsonins completely because when the washed leukocytes were suspended in salt solution and mixed with bacterial suspension no phagocytosis resulted. The bacterial suspension was made in salt solution from growths on Löffler's blood serum. The clumps were removed and the proper density obtained by centrifugation. The number of leukocytes indicated in the table was obtained by counting the leucocytes in the blood cream used in the experiment. Differential leukocyte counts were made often enough to determine that not only was the total number of leukocytes approximately the same but also that the polymorphonuclears were present in equal numbers in the blood creams used.

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[1] Ruediger, *Trans. Chicago Path. Soc.*, 1903, 6, p. 45; Hamilton, *Jour. Infect. Dis.*, 1904, 1, p. 690; Hamilton and Horton, *ibid.*, 1906, 3, p. 128.

[2] *Jour. Infect. Dis.*, 1906, 3, p. 683.

The amount of phagocytosis indicated in Table 2 was determined by counting the number of bacteria in at least 50 leukocytes and obtaining an average. The results given in Charts 1 and 2 were obtained on the dates indicated from experiments identical with the one given in Table 2.

TABLE 2.
EFFECT OF NORMAL AND PATIENT'S LEUKOCYTES AND SERUM ON PSEUDODIPHTHERIA BACILLUS.

Washed Leukocytes, Serum, and Bacillary Suspension—Equal Parts	No. of Leukocytes per c.mm	Phagocytosis per Leukocyte After 15 Min.	Remarks
Normal leukocytes+normal serum.......	15,300	4.1	No agglutination
" " +patient's serum.....	15,300	5.27	Moderate agglutination of bacilli
Patient's leukocytes+normal serum	14,700	4.2	No agglutination
" " +patient's serum....	14,700	2.05	Moderate agglutination of bacilli and leukocytes

The solid heavy line in Chart 1 represents the opsonic index as determined in the usual way, i. e., with normal leukocytes. It is above normal upon three successive days and practically normal on the first injection. The following day it rises to 1.4 to drop to 0.7 the second day after the injection; after the next two injections it remained almost normal.

The heavy broken line represents the opsonic index of the patient's serum with the patient's leukocytes. It is constantly below normal except on the first and second day after each injection, at which time it shows a sharp rise and each time going higher than the index obtained with normal leukocytes. Is this increase in phagocytosis following the injections the result of an increased activity of leukocytes or of opsonin, or of both combined? In order to decide these points, Chart 2 was prepared. Table 2 shows that by dividing the result obtained with normal leukocytes and normal serum by the results obtained with the patient's leukocytes and normal serum (Lines 1

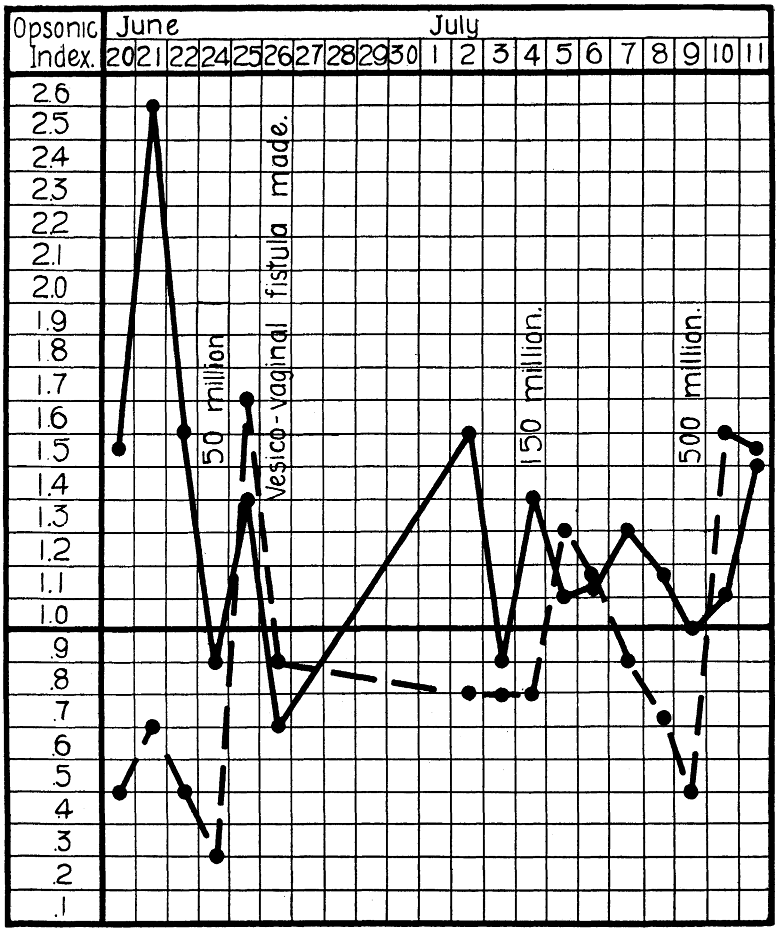

Chart 1.—Comparison of the opsonic index obtained with normal and patient's leukocytes. Solid line = Opsonic index with normal leukocytes. Broken line = Opsonic index with patient's leukocytes.

and 3) we get an idea of the phagocytic power of the patient's leukocytes as compared with normal leukocytes under the influence of normal serum. The solid line in Chart 2 gives a number of results obtained in this way and it appears that under the influence of normal serum the patient's leukocytes became less active after the second and third injections, slightly more active after the first injection. In the same way by dividing the result obtained with the patient's leukocytes and serum by the result obtained with normal leukocytes and

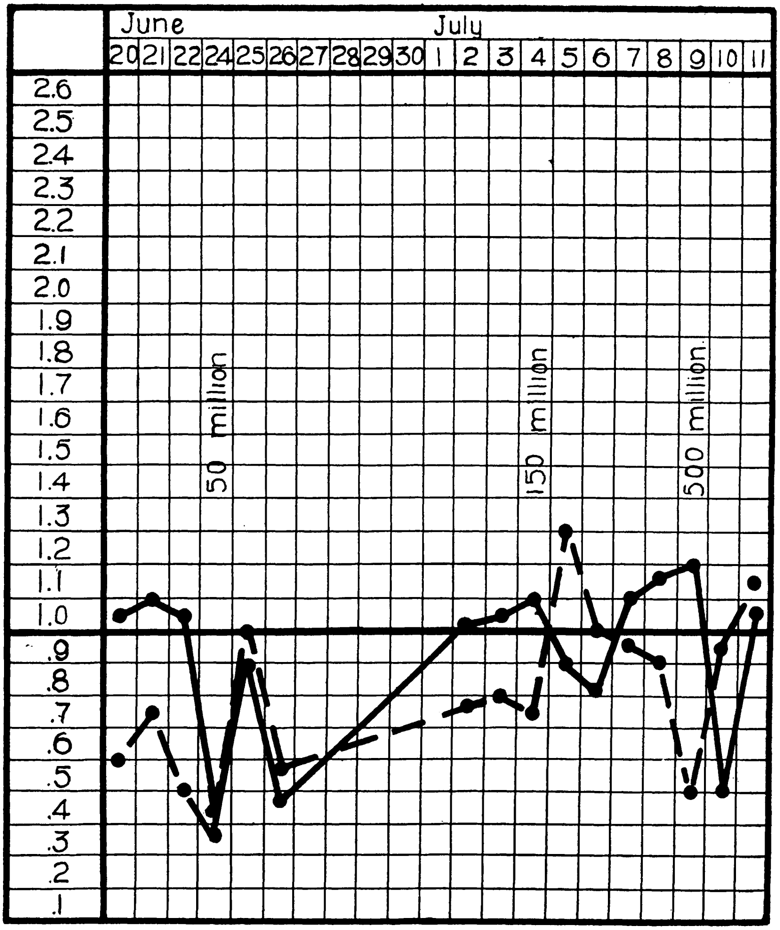

CHART 2.—Comparison of the phagocytic power of normal and patient's leukocytes. Solid line = Phagocytic power of patient's leukocytes in normal serum. Broken line = Phagocytic power of patient's leukocytes in patient's serum. (Standard of comparison—the heavy line at 1.0—is formed by the phagocytosis obtained in mixtures containing normal serum and normal leukocytes.)

serum we obtain an idea of the phagocytic power of the patient's leukocytes as compared with normal leukocytes under the influence of the patient's serum (Lines 2 and 4). All three injections appear to have been succeeded by an increased phagocytic power of the patient's leukocytes when in patient's serum (broken line, Chart 2). This reaction was specific for the bacillus injected and not obtained with pneumococci or tubercle bacilli.

To summarize, the injections were associated with (1) a rise in the opsonic power of the serum for normal leukocytes in two instances when the index was normal on the day of injection, and a drop once when the opsonic index was 1.4 at the time of injection; (2) a uniform rise in the opsonic power of the patient's serum for the patient's leukocytes; (3) a drop in the activity of the patient's leukocytes in normal serum on two occasions and a slight rise on one; (4) a uniform increase in the activity of the patient's leukocytes in the patient's serum. It is of interest to note that the injections were followed by a moderate leukocytosis. It is not unlikely that in many chronic infections the same conditions prevail as in this case, viz., that while the opsonic index in the usual sense may be quite high, yet the phagocytic power of the patient's blood (leukocytes and serum) may be below normal to the particular microbe causing the infection. In Table 2 it is noted that there occurred moderate agglutination of bacilli in the presence of the patient's serum. This was constant both before and after the injections, and the agglutination was not more marked when the index was 0.3 than when it was 1.7. Dilution experiments also gave the same results, distinct agglutination occurring at the end of eight hours in a dilution of 1:250 in the patient's serum, while in the normal serum discernible agglutination disappeared in dilutions above 1:10.

The opsonic and agglutinating power of the serum on the colon bacillus isolated from the bladder seemed to be normal.

SUMMARY.

The diphtheritic ulceration of the bladder in this case was caused by the pseudodiphtheria bacillus for the following reasons:

1. The constant presence of the bacillus in the urine and its predominance in the smears from the scrapings.

2. The specific and peculiar behavior of the blood toward this bacillus especially after the injection of dead bacteria with apparent benefit for the patient.

3. The high agglutinating power of the serum on this bacillus and absence of such power on the colon bacillus.

While the injection of killed bacteria tended to raise the power of the serum for normal leukocytes, the rise was uniformly greater for the patient's leukocytes and this seemed specific with respect to the bacterium injected and presumably the cause of the infection. The phagocytic activity of the patient's leukocytes in the presence of normal serum tended to become less after the injection, but greater when in their own serum, and this was also specific with respect to the bacillus injected. This observation suggests that too much reliance should not be placed on the opsonic index as determined in the usual way as a criterion of the actual conditions and that determination of the specific phagocytic power of the patient's blood would be of greater value because it approximates more closely actual conditions.